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NH_2-TERMINAL OF GASTRIN-17 IN DUODENAL ULCER DISEASE: IDENTIFICATION OF PROGASTRIN-17

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Serum gastrin concentrations were measured using antisera with specificity for the carboxyl and amino terminus of gastrin-17 in 50 healthy subjects and 18 patients with active duodenal ulcer disease (DU).The amino terminal of gastrin-17 immunoreactivity was significantly higher in DU patients than in healthy subjects. NH_2-terminus of gastrin-17 immunopurified material from serum of DU patients was subjected to Sephadex G50 column chromatography and eluates were monitored by an additional antiserum EG10 that recognizes COOH-terminally extended gastrin. Besides the NH_2 terminal tridecapeptide of gastrin-17, COOH-terminally extended progastrin was found. This may reflect abnormal processing of gastrin in patients with active duodenal ulcer disease.

The antral hormone gastrin is a major hormonal stimulus for gastric acid secretion, yet in duodenal ulcer disease, despite considerable evidence for gastric acid hypersecretion, there is little evidence for abnormalities in the secretion of gastrin (1,2). Hypersecretion of gastrin and ulcer disease in gastrinoma is well known (3,4). We have previously shown that gastrinoma patients secrete unusual molecular forms of gastrin (3). Whether this applies to other acid hypersecretory states is not known.

Since most of the antisera used are raised against biologically active carboxy terminal amide (Trp-Met-Asp-Phe-NH_2) region of the gastrin molecule, and gastrin precursors are carboxyl-terminally extended by a nonapeptide sequence initiated by Phe-Gly-Arg-Arg (5), the newly synthesized gastrin molecules by an abnormal processing may not be recognized. In view of this, we decided to examine the gastrin molecular heterogeneity in DU patients utilizing antisera that recognize the carboxyl and amino terminus of gastrin-17, and carboxy terminally extended gastrins.

MATERIALS AND METHODS

PATIENTS

Eighteen patients with active duodenal ulcer disease (DUs) and 50 normal

0006-291X/87 $1.50

TABLE I

Age and sex of subjects included in the study

Group	Total	Men	Women	Mean Age Years (Range)
Normal	50	27	23	46 (24-73)
Duodenal Ulcer	18	12	6	43 (14-78)

subjects were investigated. Table I gives the demographic profile of the subjects studied. Serum samples were collected from patients with DUs prior therapy during the period of acute attack, and after an overnight fast from normal subjects.

RADIOIMMUNOASSAYS

The total concentration of NH_2-terminal gastrin was measured by antiserum MG2 which is specific for the NH_2-terminal portion of gastrin-17 (3,6). The concentration of amidated carboxy terminal gastrin was estimated by using antiserum G which is specific for the amidated carboxy terminus of gastrin (6). Synthetic gastrin-17 (Becton-Dickinson, Orangeburg, New York) was used as standard and tracer (6).

Antiserum EG10 was raised in rabbits by immunization with the carboxy terminally extended fragment G11-20. Antiserum EG10 reacts equally with gastrin-17, gastrin-34 and COOH-terminally extended G11-20. It does not react with 1-13 Gastrin. Use of radiolabeled G11-20 or gastrin-17 did not alter the specificity of the assay. Fig.1 gives the recognition sites of the three antisera.

CHROMATOGRAPHY-

Serum samples from selected patients with duodenal ulcer disease were fractionated on columns of Sephadex G-50 (superfine 100 x 1 cm) at 4^{o} C. The columns were calibrated with gastrin-34, gastrin-17, and 1-13 gastrin-17. Recoveries of gastrins were(%): gastrin-34 (70 $\pm$ 3), gastrin-17 (84 $\pm$ 3) and 1-13 gastrin (81 $\pm$ 1) respectively (Mean $\pm$ SEM). The column details are given in ref. 6.

IMMUNOABSORPTION

In order to purify and establish the identity of the individual peaks measured by the NH_2-terminal RIA in gel chromatography eluates, immunoaffinity chromatography using antiserum MG2 was used. The immunoglobulins from 5 ml of antiserum MG2 were precipitated by the addition of 5 ml of saturated ammonium sulfate and then coupled to 300 mg of -CNBr activated sepharose 4B according to the manufacturer's instruction (Pharmacia, Uppsala, Sweden). The immunoaffinity studies were carried out by the method described previously (6). The immunoaffinity column binds to peptides containing the NH_2 terminus of gastrin-17 only. Recoveries of gastrin-17, 1-13 gastrin-17 and gastrin-34 were 97%, 95% and 1%, respectively.

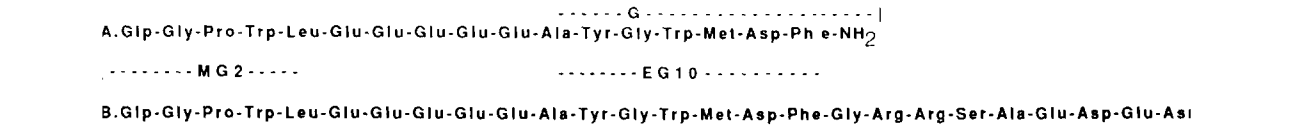


Fig. 1. The amino acid sequence of human gastrin-17 is shown in (A) and that of COOH-terminally extended progastrin-17 as deduced from gene studies is shown in (B). Braces indicate the sequence of gastrin which three antisera recognize.

RESULTS

Serum NH_2 and COOH terminal gastrin-17 concentration -

The mean fasting serum gastrin concentration as measured by antiserum MG2 specific for the NH_2-terminal of gastrin-17 in patients with DUs (n=18) was 198 ± 43 (pg/ml ± SEM) which was significantly higher than 11 ± 0.2 in healthy controls (n=50) (Table II). In contrast, there was no difference in carboxy terminal gastrin levels among groups. This is in agreement with previous findings (2,7).

COOH- and NH_2-terminal gastrin-17 molecular forms in DU patients-

The major molecular form found in patients with DU corresponded to gastrin-34 when carboxy terminal antiserum was used, but 1-13 gastrin-17 when NH_2-terminal antiserum was used (Fig. 2). In addition to these molecular forms, there were two peaks measured by the NH_2-terminal antiserum. Since these peaks were not recognized by COOH terminal antiserum, they may be COOH terminally extended progastrin-17.

Characterization of progastrin-17

As shown in Fig. 3A Sephadex G50 column chromatography of NH_2-terminally immunopurified serum from patients with DUs showed NH_2-terminal tridecapeptide and two additional peaks in the NH_2-terminal assay. There was a minor peak corresponding to gastrin-17 in the carboxy terminal assay (Fig. 3B). However, antiserum EG10 which reacts with COOH-terminally extended gastrins recognized the two additional peaks. Thus, the two peaks are likely to be COOH-terminally extended progastrin-17.

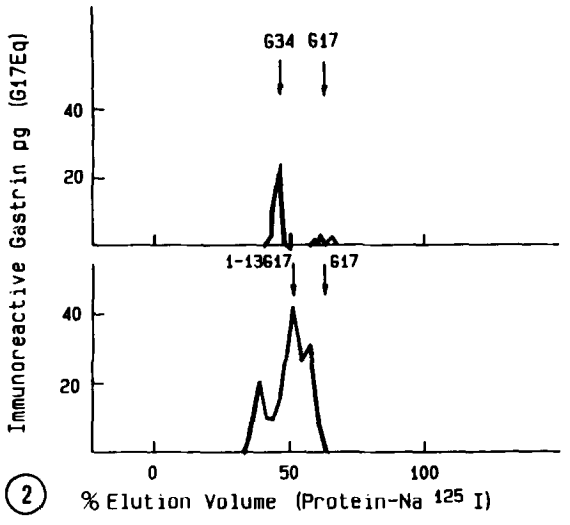
TABLE II

Immunoreactive gastrin concentration (pg/ml) in serum

	Normal	Duodenal Ulcer
	N=50	N=18
NH_2	11 ± 0.2	198 ± 43.0*
COOH	65 ± 8.0	91 ± 23.0

*Significantly different from normals.

Radioimmunoassays were performed using antiserum MG2 for the NH_2-terminus and G for the carboxy terminus of gastrin-17 measurement. The results are expressed relative gastrin-17 standard.



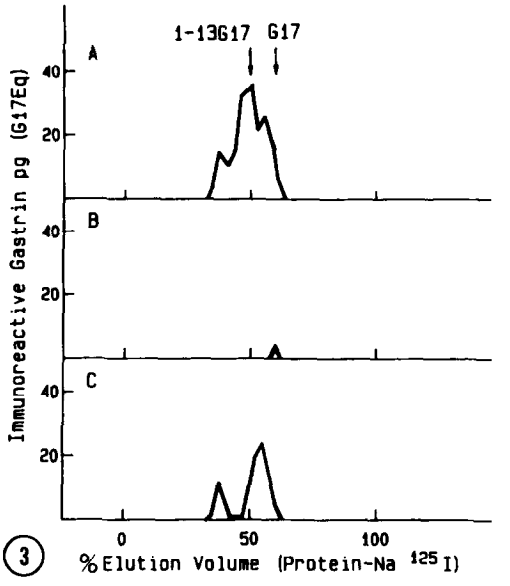


Fig. 2. Gel chromatography of amidated COOH-terminal of gastrin (upper panel) and NH_2-terminal of gastrin-17 (lower panel) in serum from DU patients. The sera were applied to sephadex G-50 superfine columns. On the ordinate gastrin immunoreactivity as gastrin-17 eq is given. On the abscissa, percent of elution volume between the protein peak (void volume 0%) and $Na^{125}I$ peak (100%) is given. Elution of synthetic gastrin-34 and gastrin-17 as measured by antiserum G (upper panel) and 1-13 gastrin-17 and gastrin-17 as measured by antiserum MG2 (lower panel) are given.

Fig. 3. Sephadex G50 column chromatography of serum from patients with DU immunopurified by an NH_2-terminally directed antiserum. Elution profiles measured by antiserum MG2 (A), antiserum G (B) and antiserum EG10 (C) are given.

DISCUSSION

The present study demonstrates that there is high concentration of NH_2-terminal tridecapeptide of gastrin-17 and progastrin-17 molecules are the major species of gastrin in the circulation of these patients. This is supported by several observations. First, progastrin-17 peaks eluted ahead of gastrin-17 in gel chromatography (Fig. 2, 3A). Secondly, none of these peaks were measured by an antiserum specific for the amidated COOH-terminus of gastrin-17 (Fig. 2, 3B). Thirdly, these peaks were detected in NH_2-terminus of gastrin-17 immunopurified material by an antiserum that recognizes COOH-terminally extended gastrins (Fig. 3C). This discriminates clearly between progastrin-17 and amidated gastrin-17.

Previous studies in DU patients have shown the predominance of gastrin-34 using COOH-terminal antiserum and NH_2-terminal tridecapeptide of gastrin-17 using NH_2-terminal antiserum (7-10). The present study using an NH_2-terminal immunoaffinity system, gel chromatography and three antisera has shown that the

material recognized by the NH_2-directed antisera used corresponds with the presence of progastrin-17.

The gastrin gene sequence (5) and a direct biosynthetic study (11) show that the formation of gastrin-17 involves processing of the dibasic residue at the carboxy-and amino-terminus extensions and subsequent conversion of -Phe-Gly at the carboxy terminus to the amidated Phe-NH_2 (Fig 1). The presence of large amounts of carboxy-terminally extended progastrin-17 and NH_2-terminus tridecapeptide of gastrin-17 in DU patients probably reflects abnormal processing of the gastrin molecule. However, this needs to be elucidated by further experiments.

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REFERENCES

1. Korman MG, Soveny C, Hansky J, (1971) Gut, 12: 899-902.
2. Trudeau WL, McGuigan JE, (1970) Gastroenterology, 59: 6-12.
3. Kothary PC, Fabri PJ, Gower W, O'Dorisio TM, Ellis J, Vinik AI, (1986) J Clin Endocrinology Metab, 62: 970-974.
4. Vinik AI, Strodel WE, Cho KE, Eckhauser FE, Thompson NW, (1983) In: Endocrine Surgery Update (Thompson NW, Vinik AI, eds.) pp 195-218. Grune & Stratton, New York.
5. Ito R, Sato K, Helmer T, Jay G, Agarwal K, (1984) Proc Natl Acad Sci USA, 81: 4662-4666.
6. Kothary PC, Vinik AI, Owyang C, Fiddian-Green RG, (1983) J Biol Chem, 258: 2856-2863.
7. Petersen B, Andersen NB, (1983) Scand J Gastroenterology, 18: 635-641.
8. Calam J, Dockray GJ, Walker R, Tracy HJ, Owens D, (1980) European J Clin Invest, 10: 241-247.
9. Petersen B, Rehfeld JF, (1980) Scand J Gastroenterology, 15: 29-31.
10. Peterson B, (1983) Scand J Gastroenterology, 18: 613-619.
11. Kothary PC, Vinik AI, (1984) Gastroenterology, 86: 1143.